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Fistulina subhepatica sp. nov. from China inferred from morphological and sequence analyses

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Abstract — *Fistulina subhepatica* sp. nov. is described from Yunnan Province, southwestern China. The main characters of *F. subhepatica* are its pileate basidiomata with rose to reddish brown pileal surface and white pore surface, individual and easily separable pores (6–9 per mm), a monomitic hyphal structure with clamped generative hyphae, and ellipsoid basidiospores (4–6 × 3–4 μm). Phylogenetic analysis inferred from the combined ITS (internal transcribed spacer region) and nLSU (the nuclear large subunit ribosomal RNA) dataset support *F. subhepatica* as a distinct new species in *Fistulina*.

Key words — *Agaricales, Agaricomycetes, Fistulinaceae*, phylogeny, rDNA, taxonomy

Introduction

Fistulina Bull. was established by Bulliard (1790: t.464) and typified by *F. buglossoides* Bull. [= *F. hepatica* (Schaeff.) With.]. It is a small but cosmopolitan genus and causes a brown rot of hardwoods. Morphologically, the genus is characterized by an annual growth habit, reddish to brown pileus surface, separate but closely packed tubes, a monomitic hyphal system, and ovoid basidiospores (Gilbertson & Ryvarden 1986, Núñez & Ryvarden 2001, Ryvarden & Gilbertson 1993).

Previous multi-gene phylogenetic studies showed that *Fistulina* fell into the euagarics clade and was closely related to *Schizophyllum* Fr., *Auriculariopsis* Maire, and *Porodisculus* Murrill (Binder et al. 2005, Bodensteiner et al. 2004).

Taxonomic and phylogenetic studies focusing on brown-rot fungi in China have been carried out recently, and some new species have been described (Cui 2013, Cui & Dai 2013, Cui & Li 2012, Cui et al. 2011, Li & Cui 2013a, Li et al. 2013, Shen et al. 2014, Zhou & Dai 2012, Zhou & Wei 2012). As a continuation of these surveys, one undescribed species of *Fistulina* was discovered based on morphological characters and phylogenetic analysis of the ITS and nLSU rRNA gene regions.

TABLE 1. List of species, vouchers, and GenBank accession numbers for sequences used in phylogenetic analysis. New sequences are indicated by bold font.

SPECIES	VOUCHER	GENBANK ACCESSION NO.	
		nLSU	ITS
Boletus satanas Lenz	Bs 2	AF336242	DQ534567
	TDB 1000c	DQ533973	
Coniophora olivacea (Fr.) P. Karst.	402	AF098376	
Favolaschia andina Singer	KG 0025	HM246679	HM246678
F. calocera R. Heim	PDD 70689	AY572006	
	PDD 71528	AY572007	
	SR.KEN. 346	AF261417	
F. cf. *calocera*	JM 98/372	AF261419	
F. peziziformis (Berk. & M.A. Curtis) Kuntze	PDD 67440	AY572008	
Favolaschia sp.	KG 0027	HM246682	HM246681
Fistulina antarctica	REG 550	AY571002	
	CBS 701.85	AY293181	DQ486702
	CIEFAP 115	AY571003	
F. hepatica	DSH 93-183	AY293182	
	REG 593	AY571004	AY571038
F. pallida	CBS 508.63	AY571005	AY571039
F. subhepatica	**Dai 11714**	**KJ925057**	
	Dai 12416	**KJ925053**	**KJ925058**
	Cui 11130	**KJ925054**	**KJ925059**
	Dai 13216	**KJ925055**	**KJ925060**
	Dai 13244 (HT)	**KJ925056**	**KJ925061**
Jaapia argillacea	CBS 252.74	GU187581	GU187524
Mycena epipterygia (Scop.) Gray	GLM 45969	AY207249	
M. galericulata (Scop.) Gray	GLM 45970	AY207251	
	TENN 7495	AF261412	
	RV 87/14.01	AF042636	
M. pura (Pers.) P. Kumm.	CBH 371	FN394630	KF913023
	IS 10/11/2000	FN394634	FN394611
	CBH 367	FN394631	KF913022
	CBH 358	FN394629	FN394608
M. rosea Gramberg	CBH 097	FN394628	FN394556
	TL 12393	FN394641	FN394555
	UP 2	FN394632	FN394550
M. sanguinolenta (Alb. & Schwein.) P. Kumm.	430360	AB512401	AB512311
Panellus edulis Y.C. Dai et al.	HMJAU 7066	GQ219739	GQ219731
	HMJAU 7214	GQ219738	GQ219730

Paxillus involutus (Batsch) Fr.	UBC F16285	EU486436	
Poromycena manipularis (Berk.) R. Heim	JM 98/217	AF261423	
Porodisculus pendulus	HHB-15376-sp	AY293204	
	DAOM 198417	AF261593	
Poromycena sp.	JM 98/128	AF261429	
Suillus cavipes (Opat.) A.H. Sm. & Thiers	TDB-646	AF071535	
Schizophyllum amplum (Lév.) Nakasone	FCUG 1803	AF141873	
S. commune Fr.	NBRC 4928	AB733339	AB733163
	NBRC 6502	AB733340	AB733164
	NBRC 30496	AB733341	AB733165
	NBRC 30749	AB733342	AB733166
S. fasciatum Pat.	CBS 267.60	AF261589	L43385
S. umbrinum Berk.	FL 02.1	AF261590	AF249391

HT = holotype

Materials & methods

Morphological studies

The studied specimens are deposited at the herbarium of the Institute of Microbiology, Beijing Forestry University (BJFC). Microscopic examinations followed Dai et al. (2010). Sections were studied at magnifications up to 1000× using a Nikon E 80i microscope and phase contrast illumination (Nikon, Tokyo, Japan). Drawings were made with the aid of a drawing tube. Microscopic features, measurements, and drawings were made from slide preparations stained with cotton blue and Melzer's reagent. Basidiospores were measured from sections cut from the tubes. In presenting the variation in the basidiospore size, 5% of measurements that were excluded from each end of the range are given in parentheses. In the text, the following abbreviations were used: IKI = Melzer's reagent, IKI– = negative in Melzer's reagent, KOH = 5% potassium hydroxide, CB = cotton blue, CB– = acyanophilous, CB+ = cyanophilous, L = mean spore length (arithmetic average of all spores), W = mean spore width (arithmetic average of all spores), Q = variation in the L/W ratios among the specimens studied, n = number of spores measured from given number of specimens. Special color terms follow Petersen (1996).

Molecular procedures and phylogenetic analysis

The Phire® Plant Direct PCR Kit (Finnzymes, Vantaa, Finland) was used to obtain PCR products from dried specimens, according to the manufacturer's instructions. A small piece of dried fungal specimen was lysed in 30 µl dilution buffer for DNA extraction. After incubating for 3 min at room temperature, 0.75 µl of the supernatant was used as template for a 30 µl PCR reaction. The internal transcribed spacer (ITS) regions were amplified with the primers ITS4 and ITS5 (White et al. 1990), and the nuclear large subunit (nLSU) ribosomal RNA gene regions with the primers LR0R and LR7 (http://www.biology.duke.edu/fungi/mycolab/primers.htm). The PCR procedure for ITS was as follows: initial denaturation at 95°C for 3 min, followed by 34 cycles at 94°C for 40 s, 54°C for 45 s, and 72°C for 1 min, and a final extension of 72°C for 10 min. The

PCR procedure for nLSU was as follows: initial denaturation at 94°C for 1 min, followed by 34 cycles at 94°C for 30 s, 50°C for 1 min and 72°C for 1.5 min, and a final extension of 72°C for 10 min. The PCR products were purified and directly sequenced in Beijing Genomics Institute, China, with the same primers. All newly generated sequences were submitted to GenBank (TABLE 1).

Sequences generated for this study were aligned with additional sequences downloaded from GenBank (TABLE 1) using BioEdit (Hall 1999) and ClustalX (Thompson et al. 1997). Prior to phylogenetic analysis, ambiguous positions at the start and the end were deleted and gaps were manually adjusted to optimize alignment. Sequence alignment was deposited at TreeBASE (http://purl.org/phylo/treebase; submission ID 15918).

Phylogenetic analysis was done as in Li & Cui (2013b). Maximum parsimony analysis and Bayesian inference (BI) was applied to the combined ITS and nLSU dataset. *Jaapia argillacea* Bres. was used as outgroup to root trees (Bodensteiner et al. 2004). The maximum parsimony tree was constructed in PAUP* version 4.0b10 (Swofford 2002). All characters were equally weighted and gaps were treated as missing data. Trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Max-trees were set to 5000, branches of zero length were collapsed and all parsimonious trees were saved. Clade robustness was assessed using a bootstrap (BT) analysis with 1000 replicates (Felsenstein 1985). Descriptive tree statistics tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated for each maximum parsimonious tree (MPT) generated.

MrMODELTEST2.3 (Nylander 2004, Posada & Crandall 1998) was used to determine the best-fit evolution model for BI. BI was calculated with MrBayes3.1.2 (Ronquist & Huelsenbeck 2003) according to the calculated model. Four Markov chains were run for 2 runs from random starting trees for 1.5 million generations, and trees were sampled every 100 generations. The first one-fourth generations were discarded as burn-in. A majority rule consensus tree of all remaining trees was calculated. Branches that received bootstrap support for maximum parsimony (MP) ≥75% and Bayesian posterior probabilities (BPP) ≥0.95 were considered as significantly supported.

Results

The combined ITS and nLSU dataset include sequences from 49 fungal collections. The dataset has an aligned length of 1388 characters including gaps, of which 812 characters are constant, 135 are parsimony-uninformative, and 441 are parsimony-informative. Maximum parsimony analysis yielded 9 equally parsimonious trees (Length = 1373, CI = 0.650, RI = 0.867, RC = 0.564, HI = 0.350), and a strict consensus tree of these trees is shown in FIG. 1. The best model for the combined ITS and nLSU partition is a GTR+I+G model. BI produced a topology similar to that from MP analysis with an average standard deviation of split frequencies = 0.007406.

In the phylogenetic tree (FIG. 1), the unknown Chinese *Fistulina* collections grouped in a well-supported lineage (MP = 88%, BPP = 1.00), which had a

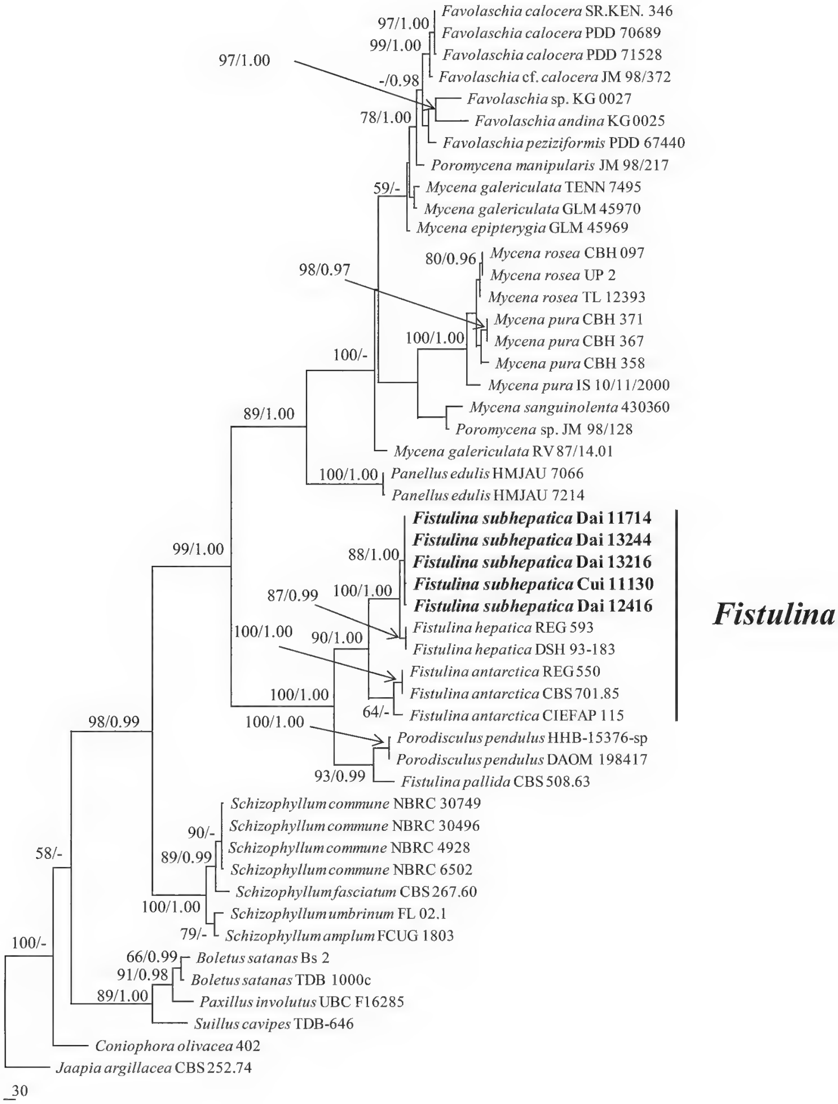
Favolaschia calocera SR.KEN. 346
97/1.00
Favolaschia calocera PDD 70689
99/1.00
Favolaschia calocera PDD 71528
97/1.00
-/0.98
Favolaschia cf. calocera JM 98/372
Favolaschia sp. KG 0027
78/1.00
Favolaschia andina KG0025
Favolaschia peziziformis PDD 67440
Poromycena manipularis JM 98/217
59/-
Mycena galericulata TENN 7495
Mycena galericulata GLM 45970
Mycena epipterygia GLM 45969
80/0.96
Mycena rosea CBH 097
98/0.97
Mycena rosea UP 2
Mycena rosea TL 12393
Mycena pura CBH 371
100/1.00
Mycena pura CBH 367
100/-
Mycena pura CBH 358
Mycena pura IS 10/11/2000
Mycena sanguinolenta 430360
89/1.00
Poromycena sp. JM 98/128
Mycena galericulata RV 87/14.01
100/1.00
Panellus edulis HMJAU 7066
Panellus edulis HMJAU 7214
Fistulina subhepatica Dai 11714
Fistulina subhepatica Dai 13244
99/1.00
88/1.00
Fistulina subhepatica Dai 13216
87/0.99
100/1.00
Fistulina subhepatica Cui 11130
Fistulina subhepatica Dai 12416
Fistulina
100/1.00
90/1.00
Fistulina hepatica REG 593
Fistulina hepatica DSH 93-183
Fistulina antarctica REG 550
100/1.00
Fistulina antarctica CBS 701.85
98/0.99
100/1.00
64/-
Fistulina antarctica CIEFAP 115
Porodisculus pendulus HHB-15376-sp
Porodisculus pendulus DAOM 198417
93/0.99
Fistulina pallida CBS 508.63
Schizophyllum commune NBRC 30749
90/-
Schizophyllum commune NBRC 30496
58/-
89/0.99
Schizophyllum commune NBRC 4928
Schizophyllum commune NBRC 6502
Schizophyllum fasciatum CBS 267.60
100/1.00
Schizophyllum umbrinum FL 02.1
79/-
Schizophyllum amplum FCUG 1803
100/-
66/0.99
Boletus satanas Bs 2
91/0.98
Boletus satanas TDB 1000c
89/1.00
Paxillus involutus UBC F16285
Suillus cavipes TDB-646
Coniophora olivacea 402
Jaapia argillacea CBS 252.74
30

FIGURE 1. The phylogenetic position of *Fistulina subhepatica* based on ITS+nLSU sequence data. Topology is from maximum parsimony analysis. Bootstrap values ≥50% and Bayesian posterior probabilities ≥0.95 are indicated at the nodes.

sister relationship with *F. hepatica* [= *F. buglossoides*, the generic type]. The new species is described on the next page.

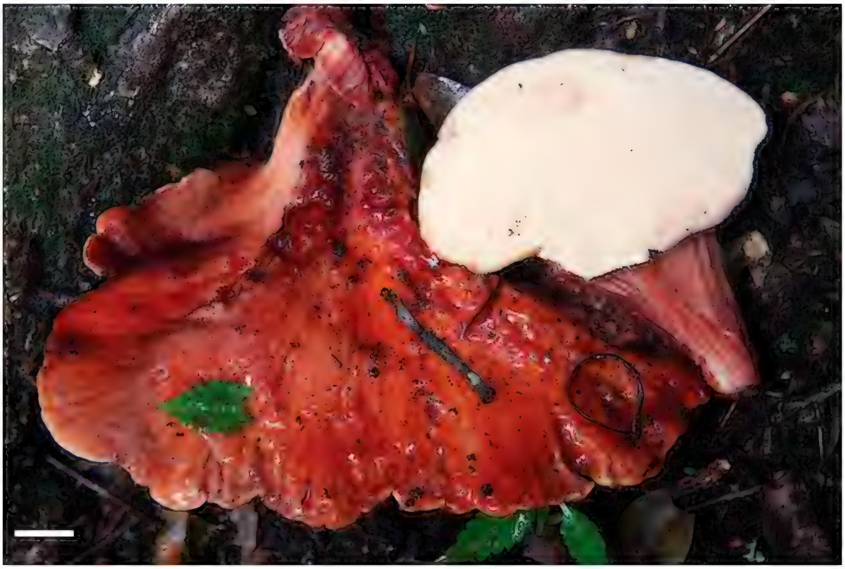

Figure 2. *Fistulina subhepatica* (holotype), basidiomata in situ. Scale bar = 2 cm.

Taxonomy

Fistulina subhepatica B.K. Cui & J. Song, **sp. nov.** Figs. 2–3

MycoBank MB 809111

Differs from *Fistulina hepatica* by its thin- to slightly thick-walled tramal generative hyphae with clamp connections and larger basidiospores.

Type: China, Yunnan Province, Jingdong County, Ailaoshan Nature Reserve, on dead tree of *Lithocarpus* (*Fagaceae*), 12.VII.2013, Dai 13244 (**holotype**, BJFC 014732; GenBank KJ925056, KJ925061).

Etymology: *subhepatica* (Lat.): refers to the morphological similarity to *Fistulina hepatica.*

Fruitbody — Basidiomata annual, pileate, fleshy, and readily exuding a reddish blood-like sap when squeezed or bruised when fresh, leathery when dry. Pileus sessile to laterally substipitate, dimidiate to reniform or subcircular, projecting up to 25 cm, 30 cm wide, and 6 cm thick at base. Pileal surface rose to reddish brown when fresh, becoming fuscous to black upon drying, faintly radially furrowed when fresh; margin acute, concolorous. Pore surface white when fresh, turning darker when bruised and becoming cinnamon-buff to reddish brown when dry; pores 6–9 per mm, consisting of individual, crowed but easily separable tubes. Context clay-buff to black, leathery when dry, up to 5 cm thick. Tubes concolorous with pore surface, leathery, up to 1 cm long.

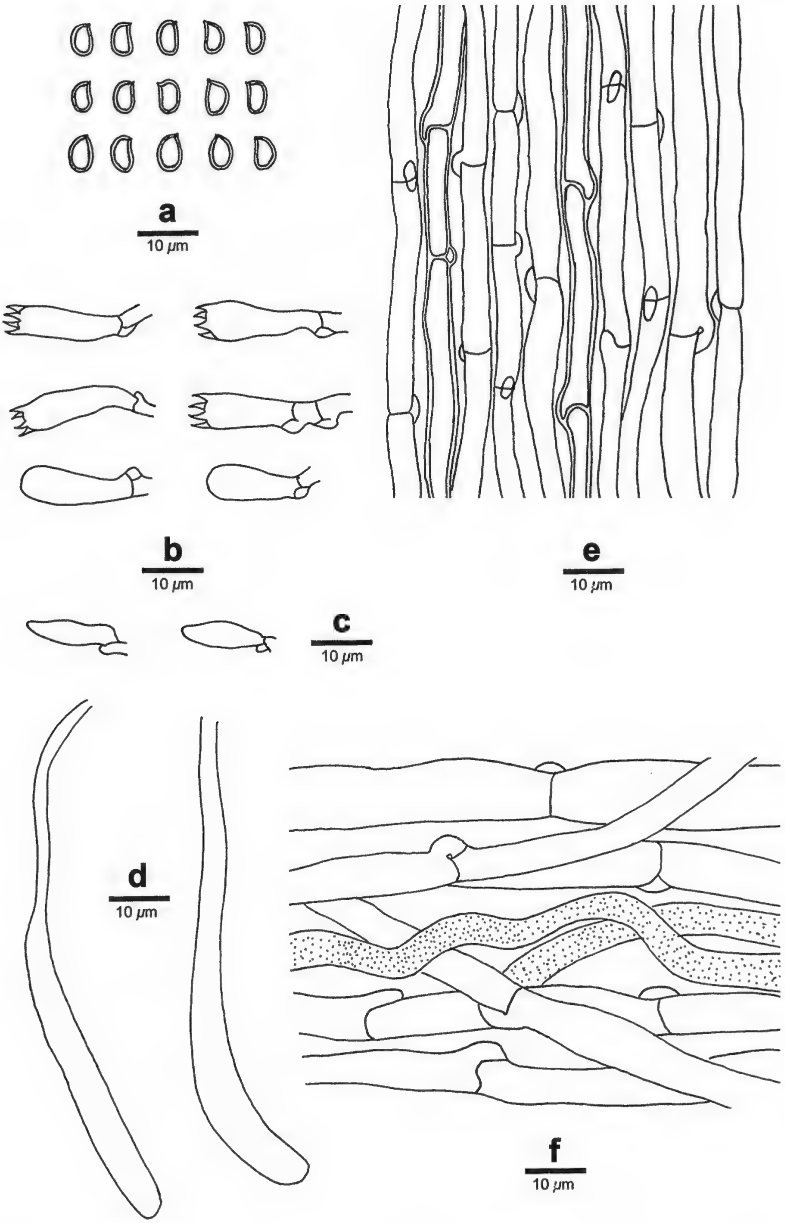

Figure 3. *Fistulina subhepatica* (holotype). a: Basidiospores. b: Basidia and basidioles. c: Cystidioles. d: Cystidial elements from dissepiment edges. e: Hyphae from trama. f: Hyphae from context. Scale bars = 10 µm.

Hyphal structure — Hyphal system monomitic; generative hyphae with clamp connections, IKI–, CB–, more or less dissolving in KOH.

Context — Generative hyphae hyaline, thin-walled, rarely branched, interwoven, 5–12 µm in diam, but with inflated portions up to 18 um, gloeoplerous hyphae present.

Tubes — Generative hyphae hyaline, thin- to slightly thick-walled, rarely branched, parallel along the tubes and densely in sections from dried specimens, 3.5–8 µm in diam. Basidia clavate with four sterigmata and a basal clamp connection, 15–32 × 5–7 µm; basidioles in shape similar to basidia, but slightly smaller. Cystidial elements present in the dissepimental edges, hyaline, thin-walled, 75–90 × 5–7 µm.

Spores — Basidiospores ellipsoid, hyaline, thick-walled, smooth, IKI–, CB+, 4–6 × 3–4(–4.5) µm, L = 4.82 µm, W = 3.37 µm, Q = 1.36–1.52 (n = 150/5).

Additional specimens examined — **CHINA. Yunnan Province**, Lincang, Xiaodaohe Forest Farm, on dead tree of *Castanopsis* (*Fagaceae*), 10.VII.2013, Dai 13216 (BJFC 014706; GenBank KJ925055, KJ925060); Nanhua County, Dazhongshan Nature Reserve, on fallen angiosperm trunk, 15.VII.2013, Cui 11130 (BJFC 015245; GenBank KJ925054, KJ925059); Chuxiong, Zixishan Forest Park, on dead tree of *Lithocarpus*, 28.VIII.2010, Dai 11714 (BJFC 008827; GenBank KJ925057); 11.VI.2011, Dai 12416 (BJFC 010696; GenBank KJ925053, KJ925058).

Other specimen examined — *Fistulina hepatica*. **FINLAND. Vantaa**, Tammosto Nature Reserve, on dead tree of *Quercus* (*Fagaceae*), 8.IX.2012, Dai 12874 (BJFC 013153).

Discussion

The previous reports of *Fistulina hepatica* in China (Dai 2012, Dai et al. 2011, Wang et al. 2011, Yuan & Dai 2008) were from identifications based only on morphological characters. According to the current phylogenetic analysis and more careful morphological examinations, these Chinese samples are newly described as *F. subhepatica*. Macroscopically, *F. hepatica* is quite similar to *F. subhepatica* in its pinkish brown to more reddish or purplish brown pileal surface, white pore surface when fresh, and individual pores; microscopically, however, *F. hepatica* differs from *F. subhepatica* by having smaller basidiospores, simple septate and thin-walled trama generative hyphae (Gilbertson & Ryvarden 1986, Núñez & Ryvarden 2001, Ryvarden & Gilbertson 1993). Moreover, *F. hepatica* forms a distinct lineage separate from *F. subhepatica* (Fig. 1). In addition, *F. hepatica* is widely distributed in temperate Europe and grows mainly on *Quercus* and *Castanea*, whereas *F. subhepatica* is found in subtropical China, occurring mostly on *Castanopsis* and *Lithocarpus*. We did not find *F. hepatica* on *Quercus* in northern China, although the big and old oak trees are suitable for the fungus.

Fistulina antarctica Speg., closely related to *F. subhepatica* in the phylogenetic analysis, differs morphologically from *F. subhepatica* by its longer basidiospores (5–7 × 3–4 µm, Spegazzini 1887).

Fistulina pallida Berk. & Ravenel resembles *F. subhepatica* in its brownish pore surface and individual pores; however, it produces smaller basidiomata and wood-brown pileus surface (Gilbertson & Ryvarden 1986). Phylogenetically, *F. pallida* falls outside the "core *Fistulina* clade" and groups with *Porodisculus pendulus* (Fr.) Murrill.

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